Michaud JP, Grant AK. 2003. Sub-lethal effects of a copper sulfate fungicide on development and reproduction in three coccinellid species. 6pp. *Journal of Insect Science*, 3:16, Available online: insectscience.org/3.16

Journal of Insect Science

insectscience.org

# Sub-lethal effects of a copper sulfate fungicide on development and reproduction in three coccinellid species.

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Received 29 April 2003, Accepted 27 May 2003, Published 13 June 2003

## Abstract

Copper-based fungicides reliably control various foliar diseases in citrus production, although they are suspected to exacerbate mite problems through various mechanisms. Studies have shown negative effects of various copper formulations on entomopathogenic fungi, nematodes, and parasitoids, but few have sought to measure its impact on the biology of predatory insects. We exposed the larvae of three species of ladybeetle (Coleoptera: Coccinellidae) to field rates of copper sulfate in combination with petroleum oil, a formulation commonly applied in Florida citrus. First instar larvae of *Curinus coeruleus* Mulsant, *Harmonia axyridis* Pallas, and *Olla v-nigrum* Mulsant received a 24 h exposure to residues on Petri dishes, and another 24 h exposure in the third instar. Treated larvae of all three species survived to adulthood at the same rate as control larvae, but larvae of *O. v-nigrum* experienced a significant increase in developmental time. Female adults of *C. coeruleus* and *H. axyridis* receiving copper sulfate exposures as larvae did not differ from control adults in pre-reproductive period, fecundity or fertility over ten days of reproduction. Treated *O. v-nigrum* females had significantly longer pre-reproductive periods than control females and laid significantly fewer eggs, although egg fertility was equivalent. We conclude that copper-sulfate fungicides are unlikely to disrupt biological control processes in citrus groves that are mediated by these coccinellid beetles.

*Keywords:* copper sulfate, *Curinus coeruleus*, development, *Harmonia axyridis*, *Olla v-nigrum*, reproduction

## Introduction

Citrus production has long relied on copper-based fungicides for disease control (Winston *et al*., 1923) and copper is still the most widely used fungicide in Florida citrus (McCoy *et al*., 2003). Cost is an important factor in the selection of control measures, and copper fungicides remain cheap and effective relative to many alternatives. Modern strobilurin fungicides such as azoxystrobin and fenbuconazole are also effective against many of the diseases controlled by copper-based fungicides, but concerns about resistance development limit their recommended application rate to once per year in a given grove (2003 Florida Citrus Pest Management Guide, 2003). The need to rotate these compounds with others of different modes of action frequently leads to the inclusion of copper in disease management programs.

Environmental concerns about the side effects of copper-based fungicides have addressed their potential for accumulation in soils and effects on soil biota such as earthworms (Paoletti *et al*., 1994) and nematodes (Jaworska and Gorczyca, 2002). Some studies have demonstrated adverse impacts on beneficial mite species (Buschkovskaya, 1974; Childers *et al*., 2001), although other studies have found no negative effects (Reis *et al*., 1999). In citrus, copper fungicide applications have been associated with outbreaks of the citrus red mites, *Panonychus citri* (Tetranychidae) (Kim *et al*., 1978), and *Phyllocoptruta oleivora* (Eriophyidae) (Winston *et al*., 1923). This effect has been attributed to chemical interactions between copper and miticides such as zineb that reduce their effectiveness (Griffiths and Fisher, 1949; Johnson, 1960). However, Childers (1994) presented evidence to indicate stimulatory effects of copper on *P. oleivora* populations independent of interactions with miticides. The primary biological control of eriophyid mites in citrus is the entomopathogenic fungus *Hirsutella thompsonii* and various studies have attributed outbreaks of citrus rust mite and other eriophyids to suppression of *H. thompsonii* with copper fungicides (van Brussel, 1975; Ureta, 1980; McCoy and Lye, 1995). Nevertheless, studies on mite control in viticulture have found copper-based fungicides to be more IPM-compatible than alternatives such as carbamates in sparing predatory phytoseiid mites (Morando *et al*., 1996; Rumbos *et al*., 2000).

The potential impacts of copper on insect biology and ecology have been less well studied. Ropek and Para (2002) showed that copper fungicides inhibit the growth and infectivity of *Verticillium lecanii*, an entomopathic fungus that can be important for aphid control in citrus (Rondon *et al*., 1981; Michaud, 1999). Glowacka *et al*. (1997) studied heavy metal loads in 14 species of psyllids (Homoptera: Psyllidae) from polluted regions in Finland and found that wax and honeydew secretions were major routes of copper elimination that largely prevented significant bioaccumulation in these insects. They speculated that certain psyllid species might be important in the transfer of heavy metals to predators such as ants.

Jalali and Singh (1995) noted some mortality of *Aphytis* sp. (Hymenoptera: Aphelinidae) following a 24 h exposure to 0.2% copper oxychloride, and reduced ability to parasitize San Jose scale, *Quadraspidiotus perniciosus* (Homoptera: Diaspididae). Mani *et al*. (1995) found that exposure to copper oxychloride reduced the longevity and fecundity of the citrus mealybug parasitoid *Leptomastix dactylopii* (Hymenoptera: Encyrtidae). Similarly, Havelka and Bartova (1991) concluded that copper fungicides were not compatible with IPM programs for greenhouses because of their adverse effects on the aphid predator *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae). However, Teran *et al*. (1993) concluded that a copper sulfate fungicide was safe for two *Aphytis* parasitoids (Hymenoptera: Aphelinidae) important for scale control in citrus.

Coccinellid beetles comprise a complex of species important as predators of various homopteran pests of citrus including scales, whiteflies, aphids and psyllids. Lo et al. (1992) reported reduced scale predation by a coccinellid, *Orcus chalybeus*, when foraging on surfaces treated with a copper sulfate fungicide. Previously, Michaud (2001a) found that a combination of copper sulfate fungicide and petroleum oil caused significant mortality to the coccinellid *Cycloneda sanguinea*, whether larvae were exposed to residues or direct topical sprays. However, another coccinellid, *Harmonia axyridis* Pallas, was not similarly affected. The results indicated that copper-oil formulations may have direct negative effects on some, but not all, coccinellid species. However, possible chronic, sub-lethal effects of copper on the developmental and reproductive biology of these insects were not sought. The current study was undertaken to determine if exposure to copper sulfate could have chronic, sublethal effects on the development and reproduction of three coccinellid species important in biological control in Florida citrus, *H. axyridis*, *Curinus coeruleus* Mulsant, and *Olla v-nigrum* Mulsant. *H. axyridis* is an invasive species in Florida (Michaud, 2002a) that is nevertheless a very effective predator of aphids and other citrus pests (Michaud 1999). Although *C. coeruleus* has a limited distribution in Florida citrus, it is an effective predator of scales and psyllids (Michaud 2002b), and *O. v-nigrum* is a key predator of Asian citrus psyllid (Michaud, 2001b).

## Materials and Methods

*Insect Colonies*

We established colonies of *H. axyridis* and *O. v-nigrum* from adults that were field-collected in Polk County, Florida in May, 2002. A colony of *C. coeruleus* was established using adults field-collected in St. Lucie County, Florida in August, 2001. Adult beetles were maintained in 1 liter, wide-mouth mason jars filled with shredded wax paper and covered with muslin fabric. Beetles were fed a combination of bee pollen and frozen eggs of the flour moth *Ephestia kuehniella* (Lepidoptera: Pyralidae). Distilled water was continuously available on a cotton wick. For oviposition, females were removed from the jars and isolated in plastic Petri dishes (5.5 cm diameter. x 1.0 cm). Insects were each provided with approx. 5-20 mg. of frozen *Ephestia* eggs every 48 h and water encapsulated in polymer beads (Entomos, LLC, 4445 SW 35th Terrace, Suite 310, Gainesville, Florida 32608) every 3 days. Eggs of *H. axyridis* and *O. v-nigrum* were usually laid directly on the surface of the plastic Petri dish and were harvested daily by moving the female to a clean dish. In nature, eggs of *C. coeruleus* are laid singly in cryptic locations, so females of this species were provided with small, trimmed squares of black synthetic carpet fiber (5.0 – 7.0 mm square) into which they readily oviposited. Eggs of all three species were held in a Plexiglass incubator until eclosion 3-4 days later (*H. axyridis* and *O. v-nigrum*) or 7-8 days later (*C. coeruleus*). Newly eclosed larvae were reared in Petri dishes (as above) on a diet of frozen *Ephestia* eggs with water made available in polymer beads.

*Copper Sulfate Treatments*

Larvae of each species were treated twice, once in the first instar (24 ± 6 h old), and again in the third instar (24- 48 h following the second larval molt). A copper-sulfate – oil emulsion was formulated to correspond to the field rate of these materials normally applied in Florida citrus for control of greasy spot, *Mycosphaerella citri* (Florida Citrus Pest Management Guide, 2003). An emulsion of 0.12 % copper sulfate (40% metallic copper, Elf Atochem Corporation) and 1.0 % (by volume) petroleum oil (Sunspray® 9E, Sunoco Inc.) was prepared in de-ionized, distilled water and applied directly to the bottom of plastic Petri dishes (5.5 cm diameter x 1 cm) using a Potter Precision Spray Tower (Burkard Manufacturing Co. Ltd., www.burkard.co.uk). The copper sulfate-oil emulsion was well agitated prior to withdrawing each aliquot. Treatment dishes (n = 60 for each species) were each sprayed with 1.0 ml of the test material and control dishes (n = 60 for each species) were each sprayed with 1.0 ml of de-ionized, distilled water. Dishes were air-dried for at least 30 min and then a small measure of *Ephestia* eggs (2.0 – 3.0 mg) was added to each. A single larvae was transferred to each dish for a period of 24 h and then removed to a clean dish and provisioned with fresh food and water for the duration of larval development.

*Larval Survival and Development*

The number of larvae alive in each treatment was tallied daily until all larvae either pupated or died. Larval developmental time was calculated in days from the date of hatching to the date the larva formed a pre-pupa. Survival was determined as the percentage of larvae that emerged successfully as adults. Adults were transferred to mason jars (prepared as above for stock colonies) as they emerged, grouped separately as 'treatment' and 'control' insects for each species. Survival was compared between treatment and control groups for each species using a Chi-squared Goodness of Fit test ($P \leq 0.05$) and developmental time, by one-way ANOVA (SPSS, 1998).

*Adult Reproduction*

Following the addition of the last emerging adult to a jar, adults were left in the jar for another seven days to ensure all females were mated. On the eighth day, all insects were removed from the jar and isolated in individual Petri dishes with food and water (as above). Only females were retained for assessment of reproductive performance. In the case of *C. coeruleus* it was possible to reliably sex individuals based on external coloration. Since neither *H. axyridis* nor *O. v-nigrum* could be sexed reliably on the basis of external features, individuals of these species were confined in pairs until sex could be determined by direct observation of copula, or by oviposition. Sex ratio was tested for deviation from 50:50 using a Chi Square, Goodness of Fit test ($P \leq 0.05$).

As females began to oviposit they were numbered sequentially and their eggs collected daily (as above) for a total of ten days of reproduction. Eggs were labeled, placed in the incubator, and the number hatching recorded. The number of eggs laid was compared between treatment and control females by one-way ANOVA. The percentage of eggs hatching was compared between treatments by one-way ANOVA. As we were unable to determine the exact emergence date for individual females following their removal from jars, the female pre-reproductive period was estimated as the period from date of removal from the jar to the date of first oviposition, plus the median number of days spent in the jar. These data were also compared by one-way ANOVA. Values of $r_m$ were calculated for each species as described by Price (1997). As these values are all based on only ten days of reproduction from each female, they underestimate $r_{max}$, but are useful for comparative purposes.

**Table 2.** Mean pre-reproductive periods (± SEM) in days for adult females of three coccinellid species surviving two 24-h exposures to residues of copper sulfate and petroleum oil as larvae.

| Species | Control | Treatment | *F* | df | *P* |
|---|---|---|---|---|---|
| *Curinus coeruleus* | 22.2 ± 0.71 | 22.1 ± 0.69 | 0.020 | 1,36 | 0.888 |
| *Harmonia axyridis* | 16.0 ± 1.22 | 15.9 ± 0.92 | 0.005 | 1,46 | 0.941 |
| *Olla v-nigrum* | 12.2 ± 0.12 | 13.0 ± 0.36 | 5.533 | 1,53 | 0.023 |

**Table 3.** Mean percentage of eggs hatching (± SEM) for adult females of three coccinellid species surviving two 24-h exposures to residues of copper sulfate and petroleum oil as larvae.

| Species | Control | Treatment | *F* | df | *P* |
|---|---|---|---|---|---|
| *Curinus coeruleus* | 82.8 ± 1.5 | 85.1 ± 1.4 | 1.138 | 1,36 | 0.293 |
| *Harmonia axyridis* | 79.2 ± 1.5 | 76.2 ± 2.3 | 1.280 | 1,46 | 0.274 |
| *Olla v-nigrum* | 65.8 ± 2.8 | 66.5 ± 2.8 | 0.031 | 1,51 | 0.862 |

## Results

*Larval Survival and Development*

The percentages of larvae of each species surviving the copper sulfate and oil treatments, along with their respective mean developmental times and standard errors, are given in Table 1. One *C. coeruleus* larvae in the treatment group was accidentally killed in the course of rearing, reducing sample size to 59. Survival was not significantly different from 100% for any species in any treatment, nor were differences between treatment and control groups significant (Chi Square, $P > 0.05$ in all cases). The copper treatment extended the developmental time of *O. v-nigrum* larvae by 8.5% but had no measurable effect on the other two species (Table 1).

*Adult Reproduction*

The sex ratios of emerging adults were not significantly different from 0.5 in any treatment, nor were there significant differences between control and treatment groups (Chi square, $P > 0.05$ in all cases). Pooled sex ratios for each species were (*C. coeruleus*: 0.38; *H. axyridis*: 0.42; *O. v- nigrum*: 0.49). A total of 2, 1 and 1 adult females died during the 21-d period in control groups of *C. coeruleus*, *H. axyridis* and *O. v-nigrum*, respectively, and 3, 2, and 1 in treatment groups, respectively. None of these females yielded ten days of oviposition data so all were excluded from reproductive analyses. The mean pre-reproductive periods for control and treatment females of all three species and their standard errors are given in Table 2. The mean fecundity of adult females of all three species for ten days of reproduction are depicted in Fig. 1. Female *O. v-nigrum* receiving copper treatments as larvae laid fewer eggs than did their control counterparts ($F = 5.598$, 1,53 df, $P = 0.023$) and had their pre-reproductive period extended by 6.6%. The mean percentage of eggs hatching was not significantly different between control or treatment females for any species (Table 3). The lower percentage of *O. v-nigrum* eggs hatching relative to the other two species was partly due to some egg cannibalism by early hatching larvae of this species that could not be prevented.

$R_m$ values were calculated for control insects only in *C. coeruleus* ($r_m = 0.054$) and *H. axyridis* (0.113) as these insects did not experience any measurable effect of treatment on any life history trait. The value for *O. v-nigrum* control insects was $r_m = 0.120$, as opposed to $r_m = 0.111$ for treatment insects, as reduction of 8.1%.

## Discussion

While we did not directly measure the amounts of copper taken up by beetles in this study, the two 24-h treatments likely represented heavy exposures of copper sulfate in comparison to anything larvae of these insects would naturally encounter in process of foraging on treated leaves in a citrus grove. While residues may persist on foliage for many days following treatment, the deposits take the form of isolated spots, clearly visible by their blue

**Table 1.** Percent survival (± SEM) of control and treatment larvae of three coccinellid species surviving two 24-h exposures to residues of copper sulfate and petroleum oil and their mean developmental times (± SEM).

| | Percent survival | | $X^2$ | *P* | Developmental time (days) | | *F* | df | *P* |
|---|---|---|---|---|---|---|---|---|---|
| Species | Control | Treatment | | | Control | Treatment | | | |
| *Curinus coeruleus* | 95.0 | 85.8 | 0.736 | ns | 16.4 ± 0.15 | 16.5 ± 0.20 | 0.148 | 1,105 | 0.701 |
| *Harmonia axyridis* | 100.0 | 100.0 | 0.000 | ns | 9.2 ± 0.15 | 9.4 ± 0.07 | 1.000 | 1, 118 | 0.319 |
| *Olla v-nigrum* | 95.0 | 95.0 | 0.000 | ns | 8.2 ± 0.09 | 8.9 ± 0.15 | 16.919 | 1, 112 | <0.001 |

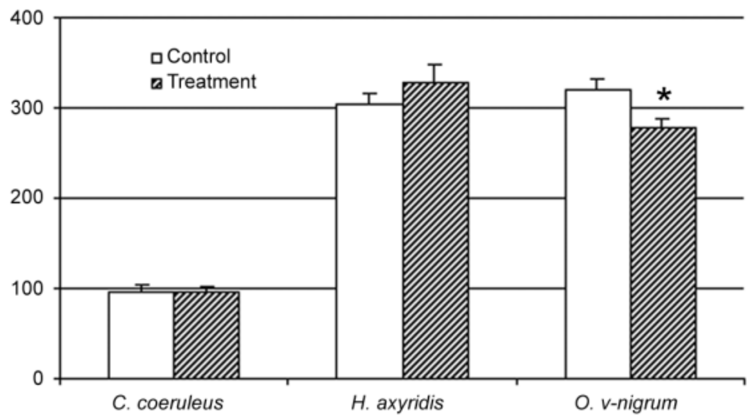


**Figure 1.** Mean numbers of eggs laid (± SEM) by adult females of three coccinellid species over 10 days of reproduction. Treatment females received two 24-h exposures to copper sulfate/petroleum oil residues as larvae. Asterisk indicates a significant difference between treatment and control groups (ANOVA, $P < 0.05$).

coloration. Consequently, insects only make sporadic contact with these deposits while foraging, as opposed to the continuous contact they had with coated surfaces in these experiments.

Three different life history parameters of *O. v-nigrum* suffered impact from larval exposures to the copper sulfate/petroleum oil formulation, whereas both *C. coeruleus* and *H. axyridis* were unaffected by the same treatments. This difference among species in apparent sensitivity to copper may arise from variation in rates of physiological uptake, elimination efficiency, or even behavioral avoidance in the case of *C. coeruleus*. The copper-oil combination seemed to exhibit some repellency to larvae of *C. coeruleus*, although not to those of *H. axyridis* or *O. v-nigrum*. Larvae of *C. coeruleus* have lower activity levels than those of the latter species (Michaud and Grant 2003) and appeared to minimize their contact with the treated surface by remaining on top of their food for extended periods. This may have reduced their uptake of the material relative to the other two species that were more active on treated surfaces. Michaud and Grant (2003) also found that third-instar *O. v-nigrum* larvae had higher activity levels than third-instar *H. axyridis* larvae, as measured by rates of larval exodus from filter paper circles. Higher activity levels may have increased exposure of *O. v-nigrum* larvae to copper sulfate during treatments. However, the provision of food on the treated surface of Petri dishes during exposure periods likely resulted in some oral ingestion of copper by all species in addition to dermal contact.

Some evidence suggests relatively inefficient uptake of copper in some insects, and effective elimination by others, factors that may explain some of the variation in the toxicity of copper across insect species. Stone *et al.* (2002) found relatively constant levels of copper in bodies of the ground beetle *Pterosthicus oblongopunctatus* (Carabidae) collected from five sites along a pollution gradient in Poland, while body burdens of zinc, lead, and cadmium all varied greatly along the gradient. Edwards and Hodgson (1973) found copper oxychloride to have no immediate toxic effects on the mite predator *Stethorus nigripes* (Coccinellidae). Mani and Thorntakarya (1988) found both Bordeaux mixture and copper oxychloride to be safe for the coccinellid *Scymnus coccivora*. Mani *et al.* (1997) found that copper oxychloride exposure had no negative effects on the longevity or reproduction of the mealybug predator *Cryptolaemus montrouzieri* (Coccinellidae). Niranjan *et al.* (1998) observed that exposure to sub-lethal doses of copper sulfate caused marked changes in the protein concentration of the hemolymph, fat bodies, and gonads of the beetle *Hydrophilous olivaceous* ((Hydrophilidae). In one of the more detailed studies of effects of copper on beetle biology, Bayley *et al.* (1995) reared larvae of *Pterostichus cupreus* (Carabidae) on copper-contaminated food and soil and measured both acute and chronic toxicity including elevated larval mortality and depressed adult locomotor functions. Izhevskii (1976) fed mineral salts to larvae of *Leptinotarsa decemlineata* (Chrysomelidae) on potato leaves and found that copper and sulfate salts depressed enzymatic activity in the beetles and delayed larval development.

The small increases in developmental time (0.7 days) and pre-reproductive period (0.8 days) observed in *O. v-nigrum* following the copper sulfate treatment were followed by a 13% reduction in female fecundity. Although these impacts on life history resulted in an 8.1 % reduction in the intrinsic rate of population increase, it seems unlikely this would translate into measurable reductions of field populations of this insect. We conclude that copper sulfate fungicides are relatively safe for field populations of these coccinellid species and are unlikely to disrupt the biological control processes in citrus groves that rely on them.

## Acknowledgements

The authors wish to thank C.C. Childers for insights and references on the history of copper usage in Florida citrus and L. Tretyak for technical support. This work was support by a grant for the U.S. Environmental Protection Agency, Region 4.

## References

Bayley M, Baatrup E, Heimbach U, Bjerregaard P. 1995. Elevated copper levels during larval development cause altered locomotor behaviour in the adult carabid beetle *Pterostichus cupreus* L. (Coleoptera: Carabidae). *Ecotoxicology and Environmental Safety* 32: 166-170.

Buschkovskaya LM. 1974. The effect of chemicals on the mite *Anystis*. *Zashchita Rastenii* 10: 53.

Childers, C.C. 1994. Effect of different copper formulations tank-mixed with fenbutatin-oxide for control of citrus rust mites (Acari: Eriphyidae) on Florida citrus. *Florida Entomologist* 77: 349-365.

Childers CC, Villanueva R, Aguilar H, Chewning R, Michaud JP. 2001. Comparative residual toxicities of pesticides to the predator *Agistemus industani* Gonzalez (Acari: Stigmeaeidae) on citrus in Florida. *Experimental and Applied Acaraology* 25: 461-474.

Edwards BAB, Hodgson PJ. 1973. The toxicity of commonly used orchard chemicals to *Stethorus nigripes* (Coleoptera: Coccinellidae). *Journal of the Australian Entomological Society* 12: 222-224.

Florida Citrus Pest Management Guide. 2003. Timmer, L.W. [ed.]. University of Florida Cooperative Extension Service, Institute of Food and Agricultural Services. Available online: http://edis.ifas.ufl.edu/

TOPIC_BOOK_Florida_Citrus_Pest_Management_Guide.

Glowacka E, Migula P, Nuorteva SL, Nuorteva P, Tulisalo E. 1997. Psyllids as a potential source of heavy metals for predators. *Archives of Environmental Contamination Toxicology* 32: 376-382.

Griffiths, J.T. and Fisher, F.E. 1949. Residues on citrus trees in Florida. *Journal of Economic Entomology* 42: 829-833.

Havelka J., Bartova P. 1991. Toxicities of greenhouse pesticides to the 3rd instar larvae of the aphidophagous gall midge *Aphidoletes aphidimyza* (Rondani) (Diptera, Cecidomyiidae). *Ochrana Rostlin* 27: 293-300.

Izhevskii SS. 1976. The physiological effect of mineral salts on the Colorado beetle, *Leptinotarsa decemlineata* Say. *Ekologiya* 4: 90-92.

Jalali SK, Singh SP. 1995. Effect of pesticide on mortality and parasitizing ability of parasitoid *Aphytis* species of San Jose scale (*Quadraspidiotus perniciosus*). *Indian Journal of Agricultural Science* 65: 617-620.

Jaworska M, Gorczyca A. 2002. The effect of metal ions on mortality, pathogenicity and reproduction of entomopathogenic nematodes *Steinernema feltiae* Filipjev (Rhabditida, Steinernematidae). *Polish Journal of Environmental Studies* 11: 517-519.

Johnson RB. 1960. The effect of copper compounds on control of citrus rust mite with Zineb. *Journal of Economic Entomology* 53: 395-397.

Kim HS, Moon DY, Lippold PC, Chang YD, Park JS. 1978. Studies on the integrated control of citrus pests. I. Bionomics of citrus red mite and natural enemies. *Korean Journal of Plant Protection* 17: 7-13.

Lo PL, Blank RH, Popay AJ. 1992. Effect of pesticides on predation of soft wax scale by the steel-blue ladybird. *Proceedings of the Forty Fifth New Zealand Plant Protection Conference*, Wellington, New Zealand, 11-13 August, 1992 pp 99-102.

Mani M, Thorntakarya TS. 1988. Studies on the safety of different pesticides to the grape mealybug natural enemies, *Anagyrus dactylopii* (How.) and *Scymnus coccivora* Ayyar. *Indian Journal of Plant Protection* 16: 205-210.

Mani M, Sushil SN, Krishnamoorthy A. 1995. Influence of some selective pesticides on the longevity and progeny production of *Leptomastix dactylopii* How., a parasitoid of citrus mealybug, *Planococcus citri* (Risso). *Pest Management in Horticultural Ecosystems* 1: 81-86.

Mani M, Lakshmi VJ, Krishnamoorthy A. 1997. Side effects of some pesticides on the adult longevity, progeny production and prey consumption of *Cryptolaemus montrouzieri* Mulsant (Coccinellidae, Coleoptera). *Indian Journal of Plant Protection* 25: 48-51.

McCoy CW, Lye BH. 1995. The effect of copper sprays on the population dynamics of the citrus rust mite, *Phyllocoptruta oleivora* (Acari: Eriphyidae) and its fungal pathogen, *Hirsutella thompsonii*. *Proceedings of the Florida State Horticultural Society* 108: 126-129.

McCoy CW, Nigg HN, Timmer LW, Futch SH. 2003. Use of pesticides in citrus IPM. In: Timmer, L.W. [ed.], 2003 Florida Citrus Pest Management Guide, University of Florida Cooperative Extension Service, Institute of Food and Agricultural Services. Available online: http://edis.ifas.ufl.edu/TOPIC_BOOK_Florida_Citrus_Pest_Management_Guide.

Michaud JP. 1999. Sources of mortality in colonies of the brown citrus aphid, *Toxoptera citricida*. *Biocontrol* 44: 347-367.

Michaud JP. 2001a. Responses of two ladybeetles to eight fungicides used in Florida citrus: Implications for biological control. *Journal of Insect Science* 1.6. Available online: insectscience.org/1.6/.

Michaud J.P. 2001b. Numerical response of *Olla v-nigrum* (Mulsant) (Coleoptera: Coccinellidae) to infestations of Asian citrus psyllid (Hemiptera: Psyllidae) in Florida. *Florida Entomologist* 84: 608-612.

Michaud JP. 2002a. Invasion of the Florida citrus ecosystem by *Harmonia axyridis* (Coleoptera: Coccinellidae) and asymmetric competition with a native species, *Cycloneda sanguinea*. *Environmental Entomology* 31: 827-835.

Michaud JP. 2002b. Biological control of Asian citrus psyllid in Florida: A preliminary report. *Entomological News* 113: 216-222.

Michaud JP, Grant AK. 2003. Intraguild predation among ladybeetles: Do the larval spines of the Chilocorinae (Coleoptera: Coccinellidae) serve a defensive function? *Bulletin of Entomological Research* (submitted).

Morando A, Morando P, Bevione D, Cerrato M. 1996. Direct and indirect effects of traditional fungicides used against *Plasmopara viticola*. *Informatore Agrario* 52: 29-32.

Niranjan C, Chanda K, Saiffullah K, Ehteshamuddin S. Choudhary N, Kumari C, Khan S. 1998. Effect of copper sulfate on protein and free amino acid concentrations of aquatic beetle *Hydrophilous olivaceous* (Hydrophilidae: Coleoptera). *Environment and Ecology* 16: 573-578.

Paoletti MG, Sommaggio D, Petruzzelli G, Pezzarossa B, Barbafieri M, Sterzynska M. 1994. 4th Seminar on Apterygota, Bialowieza, Poland, *Polskie Pismo Entomologiczne* 64: 113-122.

Price PW. 1997. Insect Ecology, Third edition. John Wiley and Sons, New York. 874 pp.

Reis PR, Sousa EO, Alves EB. 1999. Pesticide selectivity to predaceous mite *Euseius alatus* DeLeon (Acari: Phytoseiidae). *Revista Brasileiro Fruticola* 21: 350-355.

Rondon A, Arnal E, Godoy F. 1981. Behavior of *Verticillium lecanii* (Zimm.) Viegas, pathogen of the brown citrus aphid, *Toxoptera citricida* (Kirk.) in citrus orchards of Venezuela. *Agronomia Tropical* 30: 201-212.

Ropek D, Para A. 2002. The effect of heavy metal ions and their complexons upon the growth, sporulation and pathogenicity of the entomopathogenic fungus *Verticillium lecanii*. *Journal of Invertebrate Pathology* 79: 123-125.

Rumbos IC, Papaioannou SP, Markoyiannaki PD, Adamopoulos I, Lozzia C. 2000. Proceedings of the meeting on Integrated control in viticulture, held at Florence, Italy, March 1-4, 1999. Bulletin OILB SROP 23: 125-126.

SPSS. 1998. SPSS 8.0 for Windows. SPSS Inc., Chicago, Illinois.

Stone D, Jepson P, Laskowski R. 2002. Trends in detoxification enzymes and heavy metal accumulation in ground beetles

(Coleoptera: Carabidae) inhabiting a gradient of pollution. *Comparative Biochemistry and Physiology - C. Toxicology and Pharmacology* 132: 105-112.

Teran AL, Alvarez RA, Orlando CA. 1993. Effect of currently used pesticides in citrus orchards on two aphelinid parasitoids. Laboratory tests. *Journal of Applied Entomology* 116: 20-24.

Ureta SEJ. 1980. Control of the mite *Retracrus elaeis* Keifer (Eriophyidae) by means of the fungus *Hirsutella thompsonii* Fisher and the inhibition of the latter by two fungicides. *Revista Augura* 6: 25-31.

van Brussel E.W. 1975. Interrelations between citrus rust mite, *Hirsutella thompsonii* and greasy spot on citrus in Surinam. *Agricultural Research Reports* No. 842, Wageningen, Netherlands.

Winston JR, Bowman JL, Yothers WW. 1923. Bordeaux oil emulsion. *U.S.D.A. Bulletin* 1178: 1-24.